

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS

1. (*currently amended*) A method of determining the relative copy number (CN) of a first nucleotide sequence I (NucSeqI) in a sample using an amplification technique, said method comprising the steps of:

- (1) adding to the sample nucleotides, primers, polymerase, fluorescently-labeled probes and optionally, any additional reagents required for amplification, wherein the sample comprises a chromosome-derived second nucleotide sequence II (NucSeqII);
- (2) performing one or more amplification cycles to amplify the NucSeqI, wherein the sample comprises a chromosome-derived second nucleotide sequence II (NucSeqII), and carrying out the following amplification steps are carried out:
 - (a) amplifying NucSeqI is amplified,
 - (b) amplifying NucSeqII is amplified,
 - (c) amplifying a third nucleotide sequence I' (NucSeqI') corresponding to NucSeqI and present in a control sample is amplified at multiple dilutions, wherein the relationship of NucSeqI and NucSeqI' is defined as
 - (A) NucSeqI hybridizes to the complement of NucSeqI', and
 - (B) NucSeqI' hybridizes to the complement of NucSeqI, both under stringent hybridization conditions, and
- (d) amplifying a fourth nucleotide sequence II' (NucSeqII'), corresponding to NucSeqII and present in a control sample, is amplified at multiple dilutions, wherein the relationship of NucSeqII and NucSeqII' is defined as
 - (A) NucSeqII hybridizes to the complement of NucSeqII', and
 - (B) NucSeqII' hybridizes to the complement of NucSeqII, both under stringent hybridization conditions,

wherein

- (i) the ratio of concentration of NucSeqI' to the concentration of NucSeqII' is known,
- (ii) standard curves SC_I and SC_{II} comprising at least two reference points are generated by amplification of NucSeqI' and NucSeqII', respectively, at multiple dilutions,

- (iii) at least one pair of amplification reactions (a) and (b) or (c) and (d) is performed in a single container and monitored by fluorescence during amplification, and
 - (iv) NucSeqI' and NucSeqII' are localized on a single vector; and
- (3) determining from the results of the amplifications of step (2) the concentrations of NucSeqI and NucSeqII using the respective standard curves SC_I and SC_{II}, to obtain the **relative CN** of NucSeqI with respect to NucSeqII by the formula:

$$\text{Relative CN} = \frac{\text{Conc-}\text{I}_{\text{SCI}}}{\text{Conc-}\text{II}_{\text{SCII}}}$$

wherein, in said formula,

- (i) “relative CN” is the ratio of the CN of NucSeqI relative to the CN of NucSeqII in the sample;
- (ii) “Conc- I_{SCI} ” is the concentration of NucSeqI determined from standard curve SC_I; and
- (iii) “Conc- II_{SCII} ” is the concentration of NucSeqII determined from standard curve SC_{II}.

2. (*previously presented*) A method for determining the absolute CN of a nucleotide sequence NucSeqI in a sample, comprising:

- (a) determining the relative CN using the method of claim 18, and
- (b) multiplying the relative CN by the absolute CN of NucSeqII per cell.

3. (*previously presented*) A method according to claim 1, wherein at least two different NucSeqI' sequences used for measuring a corresponding number of different NucSeqI sequences are localized on a single vector.

4. (*previously presented*) A method according to claim 1 wherein the sequences of NucSeqI and NucSeqI' are the same.

5. (*previously presented*) A method according to claim 1 wherein the sequences of NucSeqII and NucSeqII' are the same.

6. (*previously presented*) A method according to claim 2, wherein at least two different NucSeqI' sequences used for measuring a corresponding number of different NucSeqI are localized on a single vector.

7. (*previously presented*) A method according to claim 2 wherein the sequences of NucSeqI and the NucSeqI' are the same.

8. (*previously presented*) A method according to claim 3 wherein the sequences of NucSeqI and the NucSeqI' are the same.

9. (*previously presented*) A method according to claim 6 wherein the sequences of NucSeqI and the NucSeqI' are the same.

10. (*previously presented*) A method according to claim 2 wherein the sequences of NucSeqII and the NucSeqII' are the same.

11. (*previously presented*) A method according to claim 3 wherein the sequences of NucSeqII and the NucSeqII' are the same.

12. (*previously presented*) A method according to claim 4 wherein the sequences of NucSeqII and the NucSeqII' are the same.

13. (*previously presented*) A method according to claim 6 wherein the sequences of NucSeqII and the NucSeqII' are the same.

14. (*previously presented*) A method according to claim 7 wherein the sequences of NucSeqII and the NucSeqII' are the same.

15. (*previously presented*) A method according to claim 8 wherein the sequences of NucSeqII and the NucSeqII' are the same.

16. (*previously presented*) A method according to claim 9 wherein the sequences of NucSeqII and the NucSeqII' are the same.

17. (*previously presented*) A method according to claim 1, wherein the sample is derived from cells.

18. (*previously presented*) A method according to claim 17, wherein an absolute CN of NucSeqII per cell is known.

19. (*previously presented*) A method according to claim 18, wherein at least two different NucSeqI' sequences used for measuring a corresponding number of different NucSeqI are localized on a single vector.

20. (*previously presented*) A method according to claim 18, wherein the sequences of NucSeqI and the NucSeqI' are the same.

21. (*previously presented*) A method according to claim 18 wherein the sequences of NucSeqII and the NucSeqII' are the same.

22. (*previously presented*) A method according to claim 19 wherein the sequences of NucSeqII and the NucSeqII' are the same.

23. (*previously presented*) A method according to claim 20 wherein the sequences of NucSeqII and the NucSeqII' are the same.

24. (*currently amended*) A method of determining the relative CN of a first nucleotide sequence I (NucSeqI) in a sample using an amplification technique, said method comprising the steps of:

- (1) adding to the sample nucleotides, primers, polymerase, fluorescently-labeled probes and optionally, any additional reagents required for amplification, wherein the sample comprises a chromosome-derived second nucleotide sequence II (NucSeqII);
- (2) performing one or more amplification cycles to amplify NucSeqI, wherein the sample comprises a second nucleotide sequence II (NucSeqII), and carrying out the following amplification steps are carried out:
 - (a) amplifying NucSeqI is amplified,
 - (b) amplifying NucSeqII is amplified,
 - (c) amplifying a third nucleotide sequence I' (NucSeqI'), corresponding to NucSeqI and present in a control sample, is amplified at multiple dilutions, wherein the relationship of NucSeqI and NucSeqI' is defined as
 - (A) NucSeqI hybridizes to the complement of NucSeqI', and
 - (B) NucSeqI' hybridizes to the complement of NucSeqI, both under stringent hybridization conditions, and
 - (d) amplifying a fourth nucleotide sequence II' (NucSeqII'), corresponding to NucSeqII and present in a control sample, is amplified at multiple dilutions, wherein the relationship of NucSeqII and NucSeqII' is defined as
 - (A) NucSeqII hybridizes to the complement of NucSeqII', and
 - (B) NucSeqII' hybridizes to the complement of NucSeqII, both under stringent hybridization conditions,

wherein

- (i) the ratio of the concentration of NucSeqI' to the concentration of NucSeqII' is known,
 - (ii) standard curves SC_I and SC_{II} comprising at least two reference points are generated by amplification of NucSeqI' and NucSeqII', respectively, at multiple dilutions,
 - (iii) at least one pair of amplification reactions (a) and (b) or (c) and (d) is performed in a single container and monitored by fluorescence during amplification, and
 - (iv) NucSeqI' and NucSeqII' are localized on a single vector; and
- (3) determining from the results of the amplifications of step (2) the concentrations of NucSeqI and NucSeqII using the respective standard curves SC_I and SC_{II}, to obtain the **relative CN** of NucSeqI with respect to NucSeqII, by the formula:

$$\text{relative CN} = \frac{\text{Conc-I}_{\text{SCI}}}{\text{Conc-II}_{\text{SCII}}}$$

wherein, in said formula,

- (a) “relative CN” is the CN of NucSeqI relative to the CN of NucSeqII in the sample;
- (b) “Conc-I_{SCI}” is the concentration of NucSeqI determined from standard curve SC_I; and
- (c) “Conc-II_{SCII}” is the concentration of NucSeqII determined from standard curve SC_{II}.